Planting depth for oilseed calendula

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ABSTRACT

Calendula (*Calendula officinalis* L.) is a popular ornamental and medicinal plant, but it also is a potential oilseed crop. Its seed oil has high levels of calendic acid, which makes it a highly valued drying oil with important industrial applications. Current agronomic information on calendula is not easily available, is limited in geographic scope, or pertains primarily to ornamental or medicinal varieties. Consequently, our objective was to investigate seedling establishment of oilseed calendula in response to planting depth and soil microclimate in field soils over two years in central Minnesota, USA. 'Carola' was used in all experiments; it is one of the few commercial oilseed varieties available. More seedlings emerged from planting depths of 1 and 2 cm than from 4 or 6 cm. Regardless of planting depth, time after planting to 50% emergence was less variable when estimated by hydrothermal time (HTT, 89 °C d, CV = 14) than calendar days (7 d, CV = 39). HTT was calculated best with a base temperature of 5.5 °C and a base water potential of −2900 kPa (−2.9 MPa). Thus, growers must plant calendula at 1–2 cm, but soil at this depth can dry rapidly, which slows accumulation of HTT and delays emergence of calendula seedlings.

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1. Introduction

Calendula (*Calendula officinalis* L., Asteraceae) is a crop with a long history of use in medicine (e.g., flos calendulae; Della et al., 1999) and ornamental horticulture (Warner and Erwin, 2005). More recently calendula is being considered as an oilseed crop, primarily because it produces a drying oil comprised largely of a special fatty acid, calendic acid (C18:3), which is in demand by the paints and coatings industry (Biermann et al., 2010).

Calendula is an annual spring-seeded plant, about 50 cm tall, and quite tolerant of low temperatures, which facilitates its production in temperate regions. Production information for ornamental and medicinal varieties of calendula is readily accessible (e.g., Angelini et al., 1997; Gomes et al., 2007; Ion, 2009; Martin and Deo, 2010; Nordestgaard, 1988; Valadares et al., 2010). However, little is known of the basic agronomic requirements of this crop for oilseed production. Although a grower's guide is available (Froment et al., 2003), the summarized information in the guide largely is based upon agronomic trials conducted in the Netherlands and United Kingdom. Only a few of these trials have been described in easily available agronomic literature (e.g., Cromack and Smith, 1998; Breemhaar and Bouman, 1995).

The 5-cm wide inflorescence of calendula is a capitulum with brilliant yellow-orange disk and ray flowers. The disk flowers develop into dry, single-seeded fruits known as "nuggets" (Froment et al., 2003). Nuggets are distinctive in appearance, being 2-mm wide tubes shaped like crescents or rings (technically called annular achenes). Diameters of whole rings are about 5 mm in the 'Carola' oilseed variety. Nuggets are grayish-brown and covered by small tubercles (warts). At first glance, nuggets are reminiscent of writhing insect larvae. Ray flowers form two other types of seeds, "wings" (cymbiform achenes) and "hooks" (rostrate achenes). The former are similar to nuggets, but with membranous tissues forming two lateral wings along the length of the crescent-shaped spatulate seeds. The width from one wing edge to the other across the middle of a seed is about 10 mm. A hook is an elongated J-shaped nugget, with a stiff beak-like tissue extending from the base of the seed, where the embryo resides, in a gently arcing manner for 10–20 mm. The morphology of the three seed types grades into one another. Detailed illustrations of seed types of *Calendula arvensis* L, which are nearly identical to those of *Calendula officinalis*, as can be seen in Ruiz de Clavijo (2005).

In *C. arvensis*, hooked and winged seeds and their embryos are about three times heavier than those of nuggets and, as might be expected, germination and seedling vigor of these former seeds are greater than for nuggets (Ruiz de Clavijo, 2005). Greater vigor allows hooked and winged seeds to emerge from greater depths than nuggets. For instance, when buried at 4 cm in soil, successful emergence of hooked, winged and nugget seeds was 84, 61, and 20%, respectively. Although comparable studies of

differing seed types have not been published for *C. officinalis*, the emergence-from-depth results for nugget seeds of *C. arvensis* match our observations for *C. officinalis*.

Despite the poorer germination and emergence of nuggets, this seed type is preferred for agronomic purposes because they are more compact and flow through equipment, such as grain drills, much better than seeds with hooks and wings. Only nuggets are recommended for commercial crops, and these should be sown 1–2 cm deep given the soil, climatic, and cultural conditions of Denmark (Nordestgaard, 1988), the Netherlands and United Kingdom (Froment et al., 2003). Sensitivity to sowing depth elsewhere is not known. Moreover, these recommendations were for older, mostly ornamental varieties of calendula whose seed oil contents are only about 5% of seed weight (e.g., Angelini et al., 1997). Sowing depth sensitivity has not been reported for newer oilseed varieties, which have considerably higher seed oil contents. For instance, seed yield samples from herbicide tolerance studies reported by Forcella et al. (in press) showed that hexane-extracted total oil content of 'Carola' seeds was $26.7 \pm 0.55\%$ (w/w, $n = 40$, personal observation). High seed oil contents are associated with enhanced seed germination, hypocotyl elongation, and a host of other vigor-related traits in *Arabidopsis* (Cernac et al., 2006). Consequently, oilseed varieties of calendula possibly have differing planting depth requirements than ornamental varieties. For these reasons we examined the sensitivity to sowing depth and soil microclimate of a recently released (2005) oilseed variety of calendula, 'Carola', in central Minnesota, USA, which has a climate, soils, and agronomic culture representative on the Upper Midwest of the USA and the southern Prairie Provinces of Canada.

2. Materials and methods

2.1. Field studies

Field trials were conducted in 2010 and 2011 at the USDA-ARS Swan Lake Research Farm (lat. 45° 41′N, long. 95° 48′W, elev. 369 m), which is located in Stevens Co., Minnesota, USA. Soil was a Barnes clay loam (Udic Haploboroll, fine mixed mesic), with about 5% organic matter and a pH of about 7.5. The previous year's crop was wheat (*Triticum aestivum* L.) in both years.

The entire experimental area both years was chisel plowed and fertilized with the equivalent of 78-33-33 kg ha⁻¹ of N–P–K, sprayed with pendimethalin (1.2 kg ai ha⁻¹), and harrowed immediately prior to seeding calendula. Nugget-type (annular) seeds of 'Carola' calendula were used in all experiments. Test weight, 1000-seed weight, and germination for these seeds were 20 ± 0.3 kg h L⁻¹, 7 ± 0.1 g, and $88 \pm 4.8\%$, respectively. Seeds were planted using a small-plot cone seeder at 11 kg ha⁻¹ (1.5 million seeds ha⁻¹) in ten rows spaced 30 cm apart on 27 May and 4 June 2010, and 4 May and 12 May 2011. The planting dates hereafter are designated as 2010 field 1, 2010 field 2, 2011 field 1, and 2011 field 2, respectively. Plots were 3 by 5 m, separated from one another by 50 cm within a block, and the three blocks (replications) were separated from one another by 5-m wide alleys. Treatments were seeding depths of 1, 2, 4, and 6 cm, which were randomized within each block.

Observations from 2010 field 1 suggested that seedling emergence was promoted in soil compacted by tractor wheels. Consequently, soil packing was added as a sub-treatment in 2010 field 2 and in both fields in 2011. Thus the experimental design was a randomized complete block with split plots. Packing was affected by a 30-cm diameter and 3-m wide steel roller-packer attached to a commercially available no-till drill. Randomized subplots were packed immediately after planting, as above. On the following day, soil cores 5 cm deep and 5 cm diameter were extracted from each

subplot, weighed, dried for 7 d at 70 °C, reweighed, and bulk density calculated on a dry weight basis.

Seedling emergence (i.e., visibly open cotyledons) was monitored three times weekly for two to three weeks by counting all calendula plants in permanently marked 1-m sections of each of two central rows within each plot or subplot. Seedling numbers (y-axis) and days after planting (x-axis) were graphed, and time to 50% emergence (t_{50}) was estimated by interpolation. Subsequently, t_{50} was converted to soil hydrothermal time (HTT$_{50}$). HTT is the duration in degree-days that soil is above a specific base temperature and base water potential, and its calculation is described elsewhere (e.g., Royo-Esnal et al., 2010; Schutte et al., 2008). Daily HTT was simulated for each soil depth using STM² software (Spokas and Forcella, 2009). The software employed common weather station variables (daily rainfall and minimum and maximum air temperatures) recorded within 100 m of the experimental sites, as well as simple soil characteristics, such as texture and organic matter content (from a site-specific database for the research farm), to estimate mean daily soil temperature and water potential at any of several user-specified soil depths. Simulated mean daily soil temperatures were converted to thermal times (TT) by subtracting a base temperature (T_b). A range of values for T_b (0–15 °C) was explored. No a priori values exist for *C. officinalis*, but Ruiz de Clavijo (2005) reported 5.5 °C for the closely related *C. arvensis*.

Similarly, daily water potentials were converted to hydro times by comparing values to a base water potential (Ψ_b). A range of Ψ_b (−10,000 to −100 kPa) was explored. This range is inclusive of Ψ_b used for many other species (Masin et al., 2010), but no prior studies have been performed on calendula.

HTT was accumulated daily, but only by the amount that daily TT exceeded T_b (otherwise TT = 0), and then only if daily Ψ exceeded Ψ_b. Cumulated mean HTT at t_{50} was determined for each seeding depth, experiment, and year ($n = 16$). Coefficients of variation (CV) of mean HTT$_{50}$ also were calculated. Tentative values for both T_b and Ψ_b were altered iteratively until CV was minimized. T_b and Ψ_b at minimum CV were considered the most effective values to use for predicting 50% emergence of calendula.

Final stand density and seed yield were determined by counting all plants and hand-harvesting all capitula along a 2-m length of each of two central rows within each plot or subplot at a time when capitula were estimated to be 70–80% brown, as recommended by Froment et al. (2003) (in commercial production, plants are desiccated chemically at the 70–80% brown capitula stage and then combine harvested, at which time seeds are at about 16% moisture (Breemhaar and Bouman, 1995)). Capitula were dried at 70 °C for one week in a forced-draft oven and then threshed. Seed samples were weighed, seed weights adjusted to 10% moisture (wet weight basis), and yields calculated on a unit area basis.

Plant densities were analyzed through ANOVA using planting depth as a fixed effect (Anonymous, 2009). Fields, years, and compaction were considered as random effects. LSD was used to separate mean densities. Seed yields were examined via nonlinear regression as functions of plant densities through the "predefined model specifications" option available in Statistix 9 software (Anonymous, 2009).

2.2. Incubator study

An incubator study was performed to examine the effect of soils with differing bulk densities on seedling emergence. Twenty 'Carola' seeds (germination ~100%) were sown at 1, 2, 4, and 6 cm depths in pots (10 cm × 10 cm) filled with either coarse sand or Barnes loam (Section 2.1). Bulk densities of these soils were 1.5 ± 0.01 g cm⁻³ for sand and 1.1 ± 0.06 g cm⁻³ for the loam. Each treatment was replicated three times. Pots were sub-irrigated, placed in an incubator set at a constant 16 °C with 12/12 h dark/light

Table 1
Time in days and soil hydro-thermal units for calendula planted at four depths to reach 50% emergence in two field trials in each of two years (2010 and 2011).

	Planting depth (cm)			
	1	2	4	6
t_{50} (d)				
2010 field 1	12.7	4.7	4.8	–
2010 field 2	4.2	4.1	4.4	4.5
2011 field 1	10.5	10.4	10.5	10.3
2011 field 2	7.0	7.5	7.4	7.2
Mean		7.3		
CV		39.4		
HTT_{50} (°C d)				
2010 field 1	90	78	82	–
2010 field 2	82	86	90	92
2011 field 1	98	103	108	109
2011 field 2	60	79	85	88
Mean		88.7		
CV		14.3		

Too few seedlings emerged to calculate t_{50} at 6 cm in field 1 during 2010. HTT_{50} values were calculated using a base soil temperature of 5.5 °C and a base water potential of −2900 kPa.

(125 μE m^{-2} s^{-1}), arranged in a randomized complete block design, and monitored daily for emerged seedlings (visible aboveground cotyledons). Seedling densities were analyzed through ANOVA using both depth and soil as a fixed effects, and LSD was used to separate means.

## 3. Results and discussion

3.1. Final seedling emergence

In the four field experiments, sowing depth affected seedling emergence ($P < 0.01$), but emergence was not influenced by fields ($P = 0.27$), years ($P = 0.07$), or packing of the soil surface ($P = 0.34$). Soil packing also had no measurable effect on bulk density (1.1 ± 0.01, 1.1 ± 0.02, and 1.1 ± 0.02 g cm^{-3} for 2010 field 2, 2011 field 1, and 2011 field 2, respectively). Consequently, data for packed and non-packed treatments, fields 1 and 2, and years 2010 and 2011 were combined. Based on the aggregated data, final seedling densities arising from seeds sown at 1, 2, 4, and 6 cm depths were 18 ± 2.6, 12 ± 1.7, 6 ± 0.9, and 6 ± 1.2 seedlings m^{-1} row, respectively (LSD = 5.1, $P < 0.01$). These densities represented about 40, 27, 13 and 13% of the sown seeds.

In the incubator experiment, final numbers of seedlings that that arose from seeds sown at 1, 2, 4, and 6 cm depths were 20 ± 1.5, 18 ± 0.3, 8 ± 0.3, and 5 ± 1.5 seedlings pot^{-1} in loam, and 18 ± 0.7, 17 ± 1.5, 1 ± 1.3, and 0 seedlings pot^{-1} in sand, respectively (LSD = 3.6 among depths and 3.5 between soils, $P < 0.01$). A soil x depth interaction ($P = 0.06$) was apparent, with more seedlings emerging in loam than sand at 4 and 6 cm depths. The lower bulk density of the loam soil may have allowed greater emergence from the deeper seeds compared to the sandy soil.

In general, both field and incubator results were aligned closely with those for emergence of nugget-like seeds of *C. arvensis* as reported by Ruiz de Clavijo (2005) for the same soil depths: 73 ± 13.2, 64 ± 13.2, 20 ± 8.7, and 0% emergence, respectively. Additionally, they matched recommendations for ornamental and medicinal varieties of calendula (Froment et al., 2003).

3.2. Time to 50% emergence

The index of emergence speed, t_{50}, was variable among experiments and sometimes among sowing depths (Table 1). For instance, 12.7 d were required to reach 50% emergence for seedlings whose
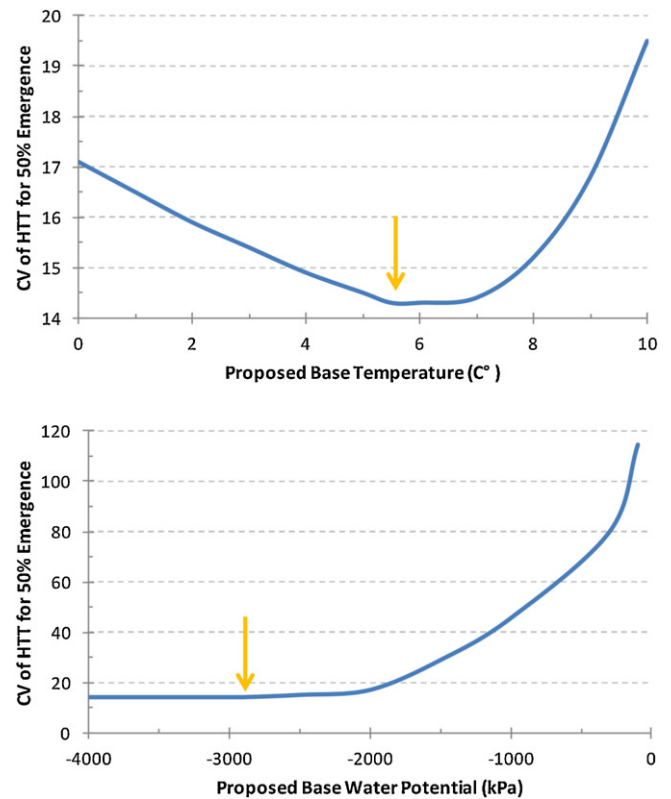
Fig. 1. Determination of base temperature and water potential for calendula through minimization of the coefficient of variability (CV) for HTT_{50} among planting depths, planting dates, and years (Table 1). Arrows indicate minima of −5.5 °C and −2900 kPa.

seeds were buried at 1 cm in 2010 (field 1) compared to 4.8 d for seedlings whose seeds were buried at 4 cm. Delayed emergence at 1 cm soil depth compared to 4 cm soil depth probably occurred because this shallow soil layer dried rapidly and often and, thereby, inhibited seed germination. Additionally, across sowing depths the range in t_{50} for 2010 field 2 was 4.1–4.5 d, whereas the range for 2011 field 1 was 10.3–10.5 d. These disparities were reflected in a mean t_{50} of 7.3 days with a relatively large CV of 39.4.

The high CV when using t_{50} could be reduced by two-thirds if HTT replaced calendar days. When using 5.5 °C as T_b and −2900 kPa as Ψ_b, then mean HTT_{50} was 88.7 and CV was 14.3 (Table 1). In essence, HTT_{50} is a much more consistent and ecologically relevant representation of the time to reach 50% calendula emergence than calendar days. However, the calculation of HTT requires species-specific values for T_b and Ψ_b. For instance, Ruiz de Clavijo (2005) selected 5.5 °C as T_b for *C. arvensis*. We examined the effect of altering T_b from 0 °C to 10 °C on the CV for HTT_{50} (Fig. 1a) while holding Ψ_b constant at −2900 kPa. CV was minimized between 5.5 °C and 6.5 °C, indicating that the most realistic base temperature for calendula likely was within that narrow range. Similarly, we examined the effect of altering Ψ_b from 0° to −4000 kPa on the CV for HTT_{50} (Fig. 1b) while holding T_b constant at 5.5 °C. CV reached a distinct inflexion point at −2000 kPa and an absolute minimum at −2900 kPa, indicating that the most realistic base water potential for calendula likely was within the range of those two values. Many other combinations of base temperatures and base water potentials were explored besides those described above, but none minimized CV below that achieved with 5.5 °C and −2900 kPa. In any event, a variable like HTT, which combines the effects of temperature and water potential, is associated with seedling emergence much more closely than calendar days after planting.

3.3. Seed yields

The relationships of calendula seed yields (Yield, kg ha^{-1}) to calendula stand densities (Stand, plants m^{-1} of row) in 2010 followed asymptotic regression equations (Anonymous, 2009):

Field 1,   Yield $= 443 - 487 \times 0.68^{Stand}$,

pseudo $R^2 = 0.94$,   SD $= 92$,   DF $= 9$

Field 2,   Yield $= 181 - 287 \times 0.81^{Stand}$,

pseudo $R^2 = 0.95$,   SD $= 56$,   DF $= 21$

Asymptotic yields were 443 and 181 kg ha^{-1} for fields 1 and 2, and these values were reached when stand densities were 18 and 25 plants m^{-1} of row, respectively.

Seed yields for 2011 were uniformly low across several calendula experiments at the Swan Lake Research Farm. Seed yields in the current experiment averaged only 24 ± 1.9 kg ha^{-1}, and they were unrelated to seeding depth or stand density, which generally was higher than in 2010 (21 ± 2.3 vs. 16 ± 2.3 plants m^{-1} of row). Seed yields can exceed 1500 kg ha^{-1} in farmers' fields in Minnesota (Forcella et al., in press). The extremely low yields in 2011 probably reflected the frequency of high air temperatures and excessive rainfall during anthesis, which likely disrupted pollination physiology or pollinator visitation. For instance, during July when anthesis occurred, maximum air temperatures exceeded 30 °C on 14 days in 2011 but only 8 days in 2010, and July rainfall was 163 mm in 2011 but only 58 mm in 2010. Calendula reproduction is highly sensitive to air temperature. For instance, in growth chambers at 32 °C compared to 20 °C, capitula numbers, sizes, precociousness, and shoot dry weight gains all were reduced greatly (Warner and Erwin, 2005). Moreover, in field settings the temperatures of capitula of C. arvensis exceeded air temperatures appreciably on sunny days, and in laboratory tests pollen germination was inhibited substantially at high temperatures (Orueta, 2002). Lastly, insect pollinators typically do not forage during periods of rain, which can lower production of entomophilous crops (e.g., Ish-Am and Eisikowitch, 1998). Combinations of these factors likely reduced calendula seed production during 2011.

Disregarding 2011, the two equations for 2010 suggest that stand densities less than about 20 plants m^{-1} of row, which are likely to occur with planting depths below 2 cm, result in reduced seed yields. Seed yields reach an asymptote at about 20 plants m^{-1} of row, which at a row spacing of 30 cm would be equivalent to 66 plants m^{-2}; i.e., the approximate target density of 60 plants m^{-2} recommended by Froment et al. (2003) for northern Europe.

4. Conclusion

Seeds of the 'Carola' oilseed variety of calendula should be planted at 1–2 cm depth to maximize seedling emergence, as previously recommended for ornamental varieties by Froment et al. (2003). However, soil microclimate at such depths is highly volatile and, after planting, these shallow soil layers may dry or change temperatures quickly to levels that inhibit seed germination and delay emergence of calendula seedlings. Regardless of the soil depth at which the seeds are planted, soil hydrothermal time of about 89 °C d must elapse for 50% emergence to occur. The appropriate base temperature and base water potential for calculation of soil hydrothermal time for calendula is 5.5 °C and −2900 kPa (−2.9 MPa). These results may assist specialty oilseed companies, their crop advisors, and prospective calendula growers to anticipate the planting and establishment requirements of calendula better, or with more assurance, than that which was possible with previously available information.

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